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AIR MAIL

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Dear Dr. Felix:

May I first express my deep appreciation for the two parcels of reprints recently received. I am delighted to have these references in my library. I was sorry to observe that your first paper on the qualitative receptor analysis was no longer available, but I am very pleasantly surprised, in fact, that you have managed to retain this set of material over this length of time.

Your letter of October 23 has put me to the strenuous, but pleasant, task of replying to your several penetrating questions. It would not be easy to summarize in a few words the various lines of evidence that have led us to the conclusion that bacteriophage particles are themselves the vectors of genetic transduction. As you may note from the paper by Lederberg, Lederberg, Zinder and Lively, in the Cold Spring Harbor Symposium, 1951, this was not the first interpretation which we put on the transduction phenomena. It was only when we discovered that bacteriophage activity was retained in full in the course of the purification of "FA" that we were led to the supposition that phage itself might be the vector. The following represents some of the particular lines of evidence which now tend to support this conclusion:

1. Every active preparation, however obtained, contains bacteriophage in a quantity commensurate with transducing activity. This quantity is of the order as indicated before of one million phage particles for each transduction of a given trait. These preparations have been made by the growth of phage as a lytic agent in the ordinary way, by the induction of lysis of lysogenic bacteria with ultraviolet light as introduced by Lwoff and somewhat to our surprise under certain conditions lysogenic bacterial cultures will release bacteriophage together with FA under the influence of such agents as penicillin and crystal-violet.

2. The concordance already mentioned of phage with transducing activity in preparations progressively purified by means of alcohol and ammonium sulfate precipitations.

3. Absence of FA from extracts prepared from bacteria without the intervention of phage, that is, from dryed cells as well as from autolysed cultures and bacteria subjected to ultrasonic followed by treatment. In one step growth experiments the appearance of FA in the filtrate is quantitatively concordant with the appearance of bacteriophage.

4. In filtration through gradocol membranes phage and FA show concordant behavior. While other methods of extraction of bacteria have failed, phages other than the original PLT 22 have been successfully applied in transduction. Thsse phages include of course the Salmonella para-typhi B phage BAOR and the Salmonella typhi phage k as I have reported to you previously. The bacteria which are capable of absorbing the transducing phage are the same types as those capable of removing FA. As has been discussed previously our present system encompasses organisms within groups B and D and to some extent A. In a few quantitative absorption experiments it has been found that the rate at which a given bacterial suspension will remove FA is concordant with the rate at which the phage is removed.

5. Similarly, the serological reactivity of phage and FA is the same. Antiserum against the phage PLT 22 inactivates the FA activity of this phage at the same rate as the bacteriophage activity is neutralized. It would be important, however, to varify the specificity of this reaction by showing that the transducing capacity of, for example, phage BAOR is neutralized by anti-BAOR serum, but not by anti-22 serum, and vice versa. We will attempt to carry out these experiments at some future time.

6. The thermal inactivation patterns of transducing activity and of bacteriophage 22 have been compared and found to be concordant. Some of these points I might mention are summarized in the paper by Zinder and Lederberg in the Journal of Bacteriology, 1951. At that time however we were not sufficiently sure of this identification of FA with bacteriophage to want to insist on this point. These arguments provide, I think, quite strong support for the notion that the vector of transduction is a particle which is released in association with phage lysis of a bacterium and which has the same size and general surface properties as that of a bacteriophage particle. The hypothesis must be considered however whether transducing particles are not in fact phage particles, but consist of particles that resemble phage in all superficial aspects while lacking the infective properties of the phage. For information on this point we must turn to some more recent experiments the burden of which is to show that the occurrence of transduction in a suitable system is regularly correlated with the induction of lysogenicity in the recipient bacterium. As I have mentioned previously, this is a correlation which often breaks down in unadapted systems, as for example, transductions from typhimurium to typhi. In other systems, however, it has been possible to show that in circumstances where the ratio of bacteriophage to bacteria in the initial infection was low that there was indeed a high correlation of transduction with lysogenization. These experiments have been carried out more thoroughly in

an Escherichia coli system than in Salmonella. But, taken together the data would seem to show that the transfer of an infectious particle is a necessary condition for transduction. As I hope I have not over-emphasized, however, this is not a sufficient condition and in other systems it is possible to see a later separation of the transduced genetic material from the phage which has carried it across.

In the light of numerous warnings from yourself and other workers, I have tried to keep as circumspect an attitude as possible concerning the specificity of H antigens in Salmonella. If our antigenic transductions were confined to a single system which might have shown initially some degree of cross-reactions, we would certainly have reserved the greatest suspicions for the reality of these results. If you entertain any lingering doubts as to the accuracy of the seratypic determinations of the newly altered types, I will be more than happy to send any number of these novel forms to you for your own examination. I do not regard myself as an expert on this subject and would be quite happy to have the benefit of your critical examination, if you should feel there were any serious virtue to such course.

I am afraid that item three of your letter opens so many questions that it would be very difficult to enter into all of their ramifications in the course of a letter of this sort. May I recommend to you that you consult Dr. C. C. Spicer on these matters as I am sure that he is thoroughly qualified to transmit my views on these questions as he learned them during the course of his visit in this laboratory not very long ago. This matter of the separation of the various levels of determination of flagella as revealed by transduction analysis would tend to be somewhat confusing and I fear that any abbreviated account that I might give here might do less good than harm. With cognizance of this risk, however, I can comment that one would refer to the restoration of motility and unmasking of a latent antigen in such cases as are represented by Salmonella typhi 0901. When motility is restored to this strain either by very infrequent spontaneous reversions selected on motility agar or by more frequent transduction of motility from other sera types the results of this reversion to motility are invariably a phase d. Thus, if phage from Salmonella typhimurium in phase i is applied to 0901 in motility medium the swarms thus engendered are invariably d. If, however, strain H901 is exposed to the same phage in the presence of anti-d serum we then find swarms produced of serotype i. This is clearly a distinct phenomenon from the uncovering of the d antigen of 0901. The unmasking of d antigen gives rise to d irrespective of the serotype of the source of the phage, whereas the experiments involving the substitution of a heterologous antigen invariably result in the formation of a flagellar type corresponding to that of the source of the phage, in this case Salmonella typhimurium where I could have compounded this illustration many fold with other strains. I may refer you on this point to table 1 of a paper by Edwards and myself which has just now appeared in the Journal of Immunology (and of which I will of course send you a reprint as soon as this is available). The type of behavior exemplified by 0901 is characteristic of most of the non-motile variant strains of Salmonella that have been studied by Stocker and myself as described in a paper now in press in the Journal of General Microbiology.

We have therefore concluded that there are genetic factors controlling the ability to produce flagella which are in general independent of other genetic factors controlling the serological type of the flagella thus produced. These remarks must be qualified however by reference to certain exceptional, and I must underline exceptional, non-motile variance. For example, the strain SW-666 derived from a monophasic S-paratyphi b. Transductions to SW-666 on motility medium generally result in two alternative types of motile forms. Those carrying the b antigen which we would regard like the other examples of transduced motility as the unmasking of the inherent b factor of this strain and a second class whose flagellar antigen is not b, but the one characteristic of the source of the phage. We have, therefore, concluded that the SW-666 transductions are exceptional in that they occasionally permit of a double transfer--one a factor for motility, two an exchange of a heterologous flagellar antigen. In genetic terminology, we would infer that we have here a pair of linked factors which may or may not be transduced together in any single event.

In connection with your item 5, I must reply that these possibilities were among the first that we considered. I am happy to report that the agglutinations by acroflavin occur quite nicely from cultures in ordinary broth and there seems now to be very little doubt but that there is in fact a true difference in the agglutinin ability of the two flagellar phases. We are very much alive to the possibilities which you had pointed out.

Some preliminary experiments have been carried out on the separation of flagella from the bacteria and to date the reactions of these isolated flagella seem to be concordant with those of the flagellated bacteria.

The "two sets of factors" to which I referred in my letter of the 12th are the two series of flagellar factors. I am afraid I do not have much hope at the present time of chemical work on the transducing genetic material itself as it seems to be inaccessibly embedded in the phage particle.

Your 7. I am pleased to be able to report that on this occasion I was in fact already familiar with the literature, that is, on the work by Hersch. I have been in touch with him as some time ago and he has courteously sent me non-motile flagellated strains. Stocker and I had also spent considerable time on a similar group of "paralized" mutants that had been isolated by Leifsid in Chicago. Our work on these mutants is described in the paper in press to which I referred to before.

May I conclude by reviewing my suggestion that you consider taking advantage of Dr. Spicer's experience in this laboratory. I am sure that the type of training that he has received would be very considerable value in developing an insight into the very vexatious questions that often arise in trying to distinguish between induction and selection of genetic alterations.

Yours very sincerely,

Joshua Lederberg
Assoc. Prof. of Genetics

JL/gw